Pre-made expression Adenovirus product manual

Catalog#	Product Name	Amounts
AVP001	**RFP** adenovirus	$1x10^8$ IFU/ml x 200ul in DMEM with 10% FBS
AVP002	**CFP** adenovirus	
AVP004	**Luciferase / GFP** adenovirus	
AVP005	**Luciferase / RFP** adenovirus	
AVP-Null	**CMV-Null control** adenovirus	
AVP001-PBS	**RFP** adenovirus, in vivo ready	$1x10^{10}$ IFU/ml x 200ul in PBS with 5% sucrose
AVP002-PBS	**CFP** adenovirus, in vivo ready	
AVP004-PBS	**Luciferase / GFP** adenovirus, in vivo ready	
AVP005-PBS	**Luciferase / RFP** adenovirus, in vivo ready	
AVP-Null-PBS	**CMV-Null control** adenovirus, in vivo ready	

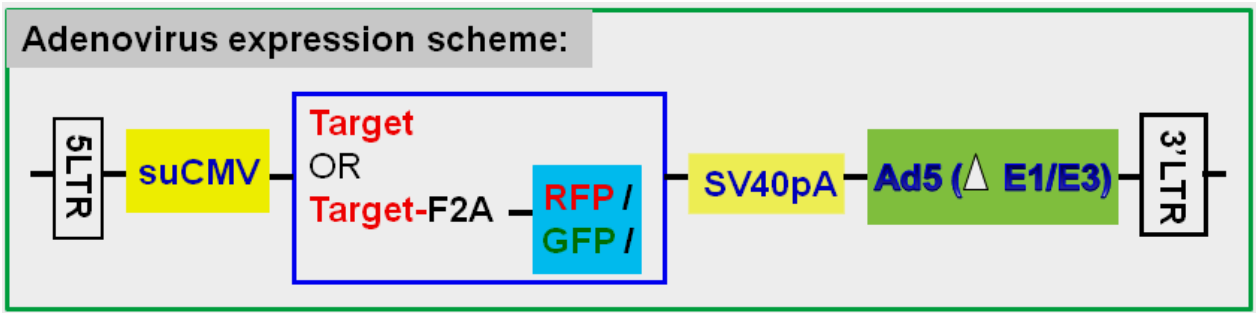
Storage: **<** -70°C, avoid repeat freeze/thaw cycles. Stable for 12 months.

Product Description:

Adenoviruses are double-stranded DNA viruses. They can infect a broad range of cell types including dividing and non-dividing cells. Thus, they are used as gene delivery vehicle. Amsbio's Adenovirus expression vector is derived from human adenovirus type5 with the E1 and E3 genomic region deleted. E1 is essential for the assembly of the virus particles. As a result, this adenovirus system only produces the replication-incompetent adenovirus. The viral replication only occurs in a cell line that provides the complementing E1 region (such as the 293A cell line) where the recombinant adenovirus are produced or amplified.

Unlike the lentivirus, adenoviral construct does not integrate into the host genome (non-insertion virus), therefore it does not activate or inactivate host genes. Once transduced into the desired mammalian cells, the recombinant target will be expressed until the viral genome is diluted out via cell division. The transgene expression decreases gradually in active dividing cells (normally in 1-2 weeks), but the expression can persist for longer time in non-dividing or low dividing cells, like skeletal muscle cell or neurons, as long as the viral genome is still present. So adenovirus is a transient expression delivery vehicle.

Amsbio pre-made expression adenovirus contains a target expressed under enhanced human cytomegalovirus (**suCMV**) promoter. When applicable, a fluorescent marker (**GFP or RFP**) is bicistronically expressed under the same promoter, mediated by a 2A element which allows expression of two individual targets. Please see map scheme below for the core expression cassette.



Note: Amsbio also provides adenovirus services for cloning of your gene of interest and generates ready-to-use adenovirus particles with the best prices and fastest turnaround time.

Key features:

1. **Pre-made, ready-to-use**: no cloning needed, no virus production. Simply add the virus to your cell line of interest for target expression.
2. **Safe to use**: the adenovirus is produced from the adenovirus vector that has the E1 and E3 gene deleted that are required to generate adenovirus, thus the adenovirus are replication-incompetent viruses.
3. **High expression level**: the target expression is driven by a super strong suCMV promoter with virus titer at $1x10^{8}$ pfu/ml (crude virus) or $1x10^{10}$ pfu/ml (concentrated virus).
4. **Efficient delivery:** the adenovirus can transduce actively dividing and non-dividing mammalian cells in culture or in vivo, and obtain close to 100% expression delivery in most cell types.

Transduction Protocols:

1. Plate your mammalian cells of choice in complete media in appropriate culture conditions and grow overnight,
2. The next day, cell density should be around 50–75% confluent. Thaw the adenoviral stock at **room temperature**, and dilute virus (if necessary, in PBS or in complete medium), or make aliquots on the first use (to save virus for next time use), directly add the appropriate amount of virus (at a suitable MOI) into cell culture. Swirl the plate gently to evenly distribute the virus, incubate at 37°C overnight;
3. On the following day (Day 2), remove the medium containing virus and replace with fresh, complete culture medium (medium change here is optional, depending on the virus toxicity on the target cells);

4. Harvest the cells (if needed) on the desired day (e.g. 2 days post transduction) and assay for expression of your recombinant protein.

Safety Precaution:

Amsbio adenovirus is replication incompetent. However, please use extra caution when using viral particles. Use the adenovirus in Bio-safety II cabinet. Wear gloves at all times when handling viral particles! Please refer to CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. Update on Adenovirus and its Vectors. J. Gen. Virol. 81, 2573-2604, 2000. Russell, W. C., et al.
2. Supernatant Rescue Assay vs. Polymerase Chain Reaction for Detection of Wild Type Adenovirus-Contaminating Recombinant Adenovirus Stocks. J. Virol. Methods 56, 99-107, 1996, Dion, L. D, et al.
3. Construction and propagation of human adenovirus vectors. In Cell Biology: A Laboratory Handbook, Ed. Celis, J. E. (Academic Press, San Diego), pp. 500–512, 1998, Hitt, M.,, et al.

Warranty and terms:

This product is for research use only. It is warranted to meet its quality as described when used in accordance with its instructions. Amsbio disclaims any implied warranty of this product for particular application. In no event shall Amsbio be liable for any incidental or consequential damages in connection with the products. Amsbio's sole remedy for breach of this warranty should be, at Amsbio's option, to replace the products.

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FAQ for pre-made Adenovirus:

1. How to use the pre-made Adenovirus? What's the control virus? What is MOI?

The pre-made Adenovirus is provided in ready to use status. Simply add certain amount of adenovirus into your mammalian cell line of choice. The amount of virus to be added is dependent on your cell types.

To obtain the desired expression level, you need to use a suitable MOI. The multiplicity of infection (MOI) is defined as the number of virus particles per cell. You should test a range of MOIs, from 0, to 1000 to determine the MOI to use. Normally, active dividing cell requires less virus amount (lower MOI). (Note: the too high MOI may kill your cells because of the toxicity of the virus or of your expressed target, the too low MOI may give low expression levels and low positive transduction rate). You should use the minimal MOI that will give 100% positive transduction rate or give you the desired expression level or transduction rate. For most cell line MOI=1-10 works great.

The Null-control adenovirus is produced from the adenovector cloned with a Null-sequence that does not express any target. The **AVP-Null** control virus (sold as separate product) serves as the negative controls for adenovirus treatment.

2. How do I know the Adenovirus is working in my cell type?

The adenovirus can transduce a majority of mammalian cells, dividing or non-dividing cells (like human, mouse, and other species) very well. However, there are few cell types can not be transduced or with very low efficiency (if so, you may try to use lentivirus). The good way to find out your cell type's susceptibility to adenovirus is to use a fluorescent control adenovirus by which you can easily visualize the fluorescent signal post transduction. Amsbio provides pre-made adenovirus expressing different fluorescent makers (GFP, RFP, YFP, CFP and fluorescent fusion markers) which can be used to test the transduction rate for your desired cell type.

3. When the target expression occurs, and how long it last?

Typically, the target expression can be detected after 12-24 hours. Depending on the cell types or dividing cycles, the expression peaks at 2-3 days post transduction, and persist for as long as the viral genome is present, from 1 week to 6 months or longer.

4. What's the buffer the virus is provided in?

Adenovirus is provided as 200ul aliquot in two formats: (1) in crude viral stock in DMEM medium with 10% serum, or (2) purified adenovirus (via column based system) and re-suspended into PBS solution with 5% sucrose for in vivo ready status.

5. Can pre-made adenovirus be used for in vivo delivery applications?

Yes. Amsbio provides adenovirus in PBS (with 5% sucrose) that is the best solution for direct in vivo applications. Please be advised, all pre-made Adenoviruses are **for research use only**, not for clinical or therapeutic applications.

6. What about the bio-safety consideration? What is RCA?

Adenoviruses are safe to use. They are non-replicable viral particles in targeting cells. They are produced from the expression vector derived from human adenovirus type5 genome in which the entire E1 (4.3kb) and E3 region was removed. E1 protein is essential for the viral replication. Without E1 protein, the packaged adenovirus cannot be replicated in target cells. The presence of the viral genome is transient and will eventually be diluted out as cell division occurs. (Note: In E1 complementing cell lines, like 293A cells, the adenovirus can be replicated or amplified).

Despite the adopted safety features, recombinant human adenovirus has been classified as biosafety level II agent, and you need BL-2 level facility to work with it. It should be noted that cell culture facilities in most institutes are certified as BL-2 level. Please refer to CDC and NIH guidelines for more details about adenovirus handling.

In rare instances, the E1 sequence from the virus production cell genome (293A cells) is integrated into adenoviral transgene construct (via sequence homologous recombination), which resulted in a replication-competent adenovirus (**RCA**). In theory, this could occur and most likely could happen in large scale virus amplification. Wild type, replication competent adenoviruses could cause cold symptoms but generally do not cause serious illness. We have not observed wild-type RCA contamination in small-scale. All Amsbio adenoviruses are made in very small scale and RCA test is not a routine test for pre-made adenovirus. For large scale adenovirus application, we measure the RCA by PCR tests. (Note: When RCA occurs, it will quickly take over the non-replicable virus, and amplified virus causes cell death). To avoid the occurrence of RCA, viruses should be produced and amplified in low passage 293 cells.

7. How is the virus titer measured?

The titers (Ifu/ml) of Amsbio's adenovirus are measured via a fluorescent marker (GFP or RFP) after the virus is transduced into HeLa cells, which are also used as titer reference for the same construct backbone containing a non-fluorescent target. The measured titer is close to the real infectious units that can be measured by a biological assay, the plaque assay, counting the PFU (plaque formation unit) after virus transduction.

8. How are the adenovirus vector constructed? Are there any antibiotic markers included in the adenovirus constructs?

Amsbio uses its proprietary Eco cloning technology (the vector built-in cloning competent cells) to directly insert a target into the E1/E3 deleted human adenovirus 5 genome.

Adenovirus is not integrated into host cell genome, therefore, it is not suitable for stable long term expression. For that reason, we do not include any antibiotic marker in the adenovirus constructs. In some cases, we have included a fluorescent marker under the same promoter for the specific target, mediated by 2A element. The fluorescent protein provides a marker for monitoring the virus performance and target expression, as well as a method of selection via fluorescent signal. For long term stable expression, please use our pre-made lentivirus for which we have included all kind of selection markers.

9. Adenovirus vs Lentivirus:

Both adenovirus and lentivirus can transduce the dividing and non-dividing cells. Both are used as expression delivery tool for mammalian cell lines or primary cells. Unlike the lentivirus, adenoviral construct does not integrate into the host genome, therefore it does not activate or inactivate host genes. For adenovirus, the transgene expression decreases gradually as cells divide. Therefore, Adenovirus delivers transient expression, whereas the lentivirus delivers stable, long term expression. Lentivirus has less immunogenicity to human cells and less toxicity than adenovirus, but adenovirus may have better transduction efficiency in most some cell types.